

Supplemental Data

Downregulation of Death-Associated Protein Kinase 1 (*DAPK1*) in Chronic Lymphocytic Leukemia

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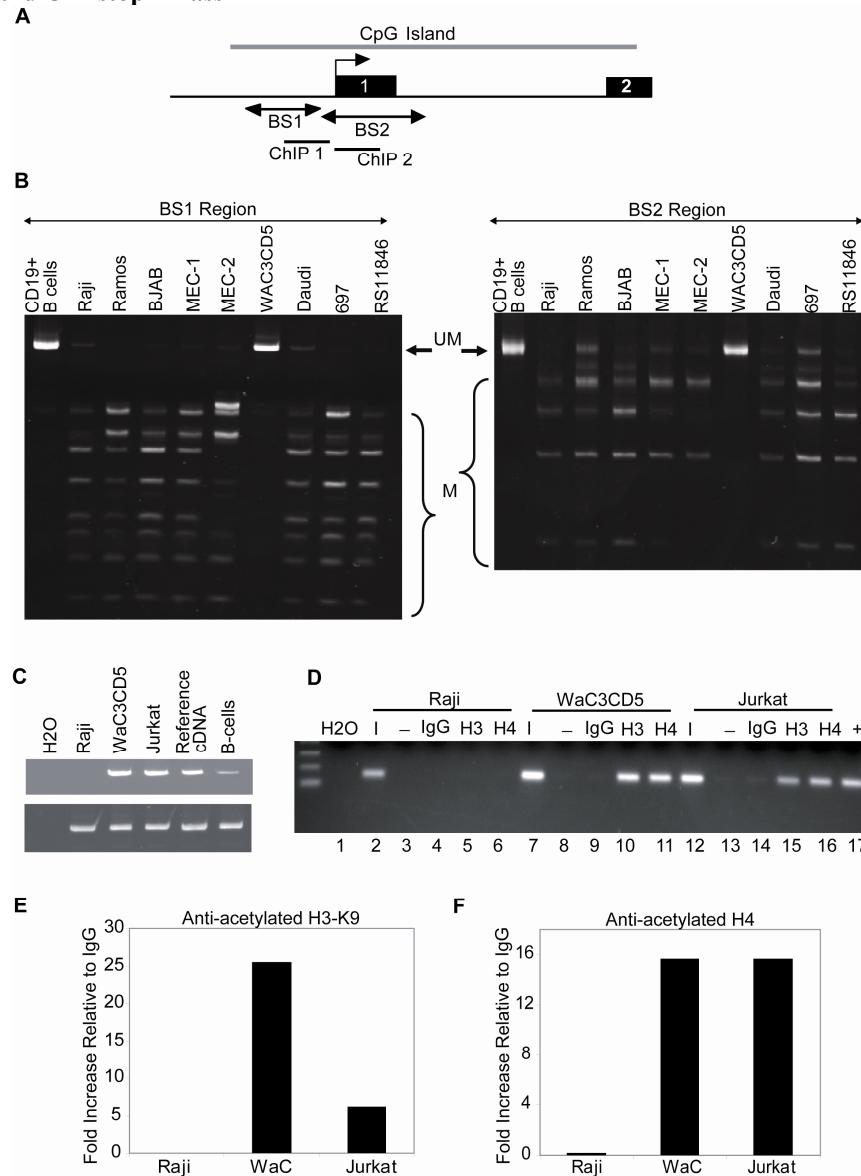


Figure S1. *DAPK1* Promoter Methylation and Histone Tail Modifications

(A) Schematic of the two regions amplified in the COBRA assay (BS1 and BS2) and in the ChIP experiments (ChIP1 and ChIP2). *DAPK1* exons 1 and 2 (black boxes) and the CpG island region frequently methylated in CLL samples (gray line) are highlighted.

(B) COBRA assays for two regions in the *DAPK1* promoter, performed on Ramos (Burkitt Lymphoma), BJAB (Atypical Burkitt Lymphoma (EBV-), MEC-1 (CLL), MEC-2 (CLL), WaC3CD5 (CLL), Daudi (Burkitt Lymphoma), 697 (ALL), RS11846 (Non-Hodgkin Lymphoma), RS 4;11 (ALL) cell line DNAs and CD19+ selected B-cells. (M) indicates digested PCR products representing the methylated portion and (UM) represents the unmethylated portion.

(C) RT-PCR for *DAPK1* and *GAPDH* expression in Raji, WaC3CD5 and Jurkat cell lines. Reference cDNA (cDNA isolated from RNA mixture of different tissue) and B-cells were used as positive control.

(D) Acetylation of histone H3 at lysine 9 and histone H4 are hallmarks of active chromatin. ChIP assay using anti-acetylated histone H3 and H4 antibody showed that in RAJI cells the *DAPK1* promoter was not associated with either ac-H3 or ac-H4 histones (lanes 5 and 6) within ChIP 2 region while WaC3CD5 and Jurkat cell lines had acetylated histones within this region (lanes 10, 11, 15 and 16). No antibody (-) or IgG was used as negative controls and input DNA (I) was the positive control.

(E and F) Quantitative ChIP assay for ChIP 1 region in Raji, Jurkat and WaC3CD5 cell lines using anti acetylated histones H3-K9 and H4 antibody. IgG was used as a negative control. The cell lines expressing *DAPK1*, (Jurkat and WaC3CD5) were enriched for ac-H3-K9 and ac-H4 in *DAPK1* promoter region, while in Raji cells, the same region lacked the markers of open chromatin. Chromatin immunoprecipitaion was carried out using the ChIP assay kit (Upstate Biotechnology, Lake Placid, NY). For immunoprecipitation, rabbit polyclonal anti-acetylated H3-K9, anti-acetylated H3 and anti-acetylated H4 antibodies (Upstate Biotechnology, Lake Placid, NY) were used. SYBR green semi-quantitative PCR was performed as described before for the quantitation. Fold difference was calculated for each cell line relative to the negative control rabbit IgG.

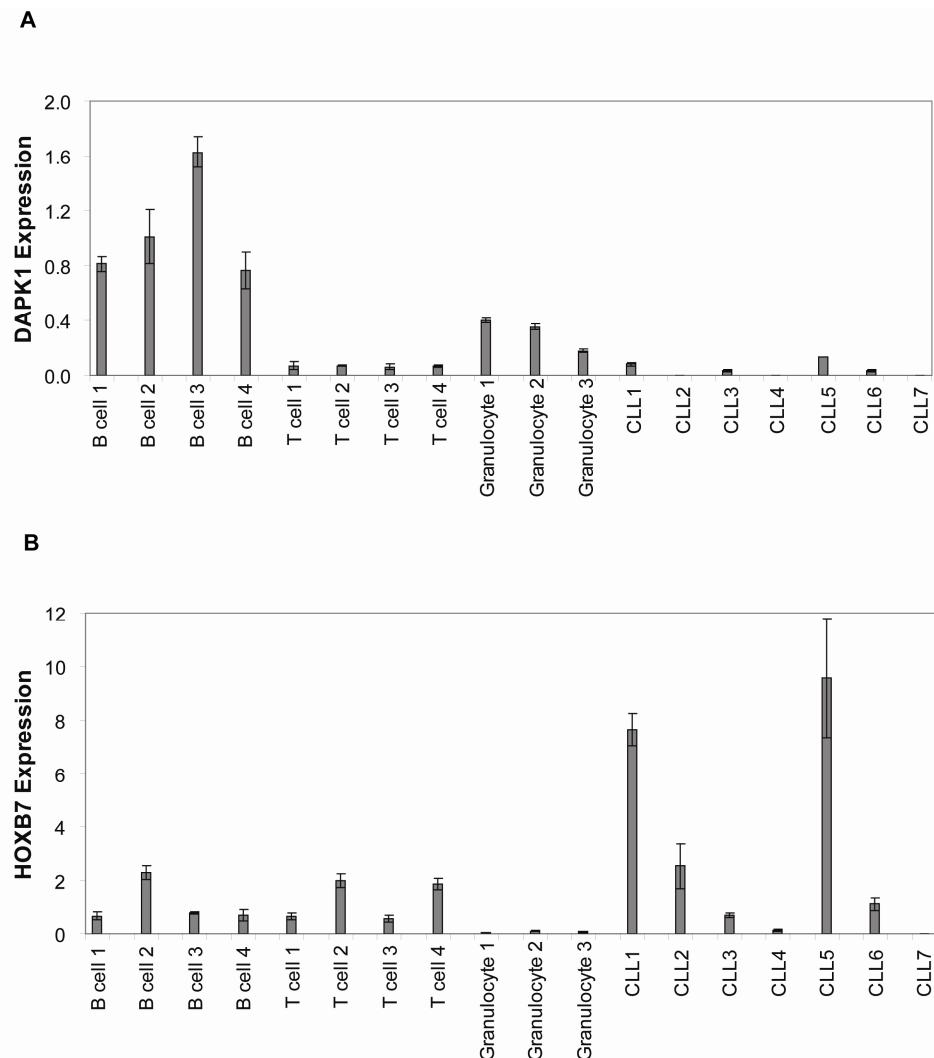


Figure S2. *DAPK1* and *HOXB7* Expression in Normal Cells and CD19 Selected CLL Cells
(A and B) Semi-quantitative RT-PCR was performed on four normal B-cells, three each of normal T-cells and granulocytes and seven selected CLL samples to study expression of *DAPK1* and *HOXB7*. *GAPDH* expression was used as an internal control.

Table S1. DAPK1 Sequencing Results

EXONS 1-26																										
Sample	l-kb	1	2	3	4	5	6	7	8	9	10	11*	12	13	14	15	16	17	18	19	20	21	22	23	24	25
WF00144																					het c.3597C>T>R					
WF00655																					het c.3150C>T>S>N					
WF0067																					het c.3597C>T>R					
WF0072																					het c.4037A>G>S>N					
WF0097																					het c.3597C>T>R>R					
WF0109																					het c.4037A>G>S>N					
WF0124																					het c.4037A>G>S>N					
JCB0091																					het c.3597C>T>R>R					
JCB0093																					het c.4037A>G>S>N					
JCB0232																					het c.3597C>T>S>N					
JGG0026																					het c.4037A>G>S>N					
JGG0072																					het c.3597C>T>R>R					
JGG0123																					het c.3597C>T>R>R					
JGG0156																					het c.4037A>G>S>N					
JGG0184																					het c.3597C>T>R>R					
JGG0229																					het c.4037A>G>S>N					
JGG0271																					het c.4037A>G>S>N					
JGG0290																					het c.3597C>T>R>R					
JGG0306																					het c.4037A>G>S>N					
JGG0372																					het c.3597C>T>R>R					
KRR0003																					het c.4043G>T>G>V					
KRR0008																					het c.3597C>T>R>R					
KRB0168																					het c.3597C>T>R>R					
KRB0235																					het c.4037A>G>S>N					
KRB0290																					het c.4037A>T>R>R					
KRB0383																					het c.4037A>G>S>N					

MJK0002			hom c.608C>T:D>D			het c.192S>A:D>D			het c.4037A>G:S>N	hom c.4037A>G:S>N	
MJK0012			het c.1608C>T:D>D						het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0041									het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0050									hom c.4037A>G:S>N	hom c.4037A>G:S>N	
MJK0059		*							het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0136									het c.4037A>G:S>N	hom c.4037A>G:S>N	
MJK0197		*							het c.4037A>G:S>N	hom c.4037A>G:S>N	
MJK0218			het c.114G>A:Q>Q			het c.1608C>T:D>D			het c.4037A>G:S>N	hom c.4037A>G:S>N	
MJK0222			hom c.114G>A:Q>Q						het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0284						hom c.1608C>T:D>D			het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0312							hom c.1608C>T:D>D		het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0379								hom c.1608C>T:D>D	het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0390									het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0403			het c.114G>A:Q>Q			het c.1608C>T:D>D			het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0424						het c.1608C>T:D>D			het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0427									het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0494									het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0566									het c.3597C>T:R>R	het c.4037A>G:S>N	
MJK0571									hom c.3597C>T:D>R	hom c.3597C>T:D>R	
MJK0583		*							het c.3597C>T:D>R	het c.4037A>G:S>N	
MJK0623						het c.1608C>T:D>D			het c.3597C>T:D>R	het c.4037A>G:S>N	
MJK0702									hom c.3597C>T:D>R	hom c.3597C>T:D>R	
MJK0735						het c.1608C>T:D>D			het c.3597C>T:D>R	het c.4037A>G:S>N	
MJK0745						het c.1608C>T:D>D			het c.3597C>T:D>R	het c.3597C>T:D>R	
MJK0771						hom c.1608C>T:D>D			het c.3597C>T:D>R	het c.3597C>T:D>R	
MRG0039		*				het c.1608C>T:D>D					
MRG0040										hom c.3597C>T:D>R	
NEK0051									hom c.4037A>G:S>N	hom c.4037A>G:S>N	
NEK0153									het c.4037A>G:S>N	het c.4037A>G:S>N	
TJK0018									het c.3597C>T:R>R	het c.4037A>G:S>N	
TJK0070									het c.4037A>G:S>N	het c.4037A>G:S>N	
TJK0093									hom c.4037A>G:S>N	hom c.4037A>G:S>N	
TJK102									het c.3150C>T:S>S	het c.3597C>T:R>R	
TJK1018									het c.3597C>T:R>R	het c.3597C>T:R>R	

TJKU0190				*					het c.3150 C>T>S het c.3597 C>T>R
TJKU0192			het c.114 G>A;Q>Q						het c.4037 A>G;S>N
TJKU0201									hom c.3597 C>T>R
TJKU0207									hom c.3597 C>T>R
TJKU0211									hom c.3597 C>T>R
TJKU0454									hom c.3597 C>T>R
TJKU0462				*					het c.3597 C>T>R
TJKU0491				*					het c.3167 C>T>V
WGWN0003				*					hom c.3597 C>T>R
WGWN0004			het c.114 G>A;Q>Q	*					hom c.3597 C>T>R
WGWN0037									hom c.4037 A>G;S>N
WGWN0052									hom c.3597 C>T>R
M1 FBL				*					het c.3597 C>T>R
M2 FBL				*					het c.3597 C>T>R
WAC									het c.4037 A>G;S>N
									hom c.3597 C>T>R

The following SNPs have been identified as common polymorphisms: C-1190>G = rs1755986; C-1608>T = rs3818584; C-1445>A = rs1755987.